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Increase of Deoxynivalenol during the malting of naturally *Fusarium* infected Chinese winter wheat

Zhao Jin [a, c], Yan Cao [b], Aimei Su [a], Yongchang Yu [a], Minwei Xu [c, *]

[a] Department of Environment and Bioengineering, Nantong College of Science and Technology, Nantong 226007, Jiangsu, China
[b] Food Science Institute, Zhenjiang Academy of Agricultural Sciences, Hangzhou 310021, Zhejiang, China
[c] Department of Plant Sciences, North Dakota State University, PO Box 6050, Dept. 7670, Fargo, ND 58108, United States

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ABSTRACT

Wheat constitutes a significant adjunct ingredient for beer brewing in China, but is prone to be contaminated with Deoxynivalenol (DON). To evaluate the development of DON and its producing-*Fusariums* in the malting, thirteen Chinese winter wheat samples of natural DON levels under the national standard limit (1000 μg/kg) were selected to malt in lab-scale. DON levels increased in the malting for all samples by 282% on average, and exceeded of 1000 μg/kg in 7 malts. Fate of DON and *Fusariums* in the malting was monitored for three samples with the relatively large increase of DON levels. Around 95% of initial wheat DON was washed away by steeping, but the DON level rose up to 7-fold in the following germination, which was caused by the growth of *Fusariums* with tri5 DNA level increasing by 28-fold averagely. Fifty-one percent of tri5 DNA in the germinated wheat was terminally denatured by high temperature in the kilning stage, but DON survived. About 26% of DON was get rid by de-rooting. The result highlighted the risk of DON increase in the malting of Fusarium Height Blight wheat.

1. Introduction

Wheat (*Triticum aestivum* L.) is a significant adjunct for the beer brewing in China which accounts for a quarter of the total output in the world. Malting barley is the traditional major grain ingredient for beer brewing, but it is excessively dependent on the import, due to the diminishing planting area in China. Alternatively, wheat is used as part of the beer brewing ingredient, based on the similar substance components with barley. Wheat beer is characterized by the rich foam and the light taste of flavors. Wheat has been historically used in the beer brewing in the world, which is famous in Southern Germany, Belgium, America and other countries (Hieronymus, 2010). For beer brewing, grain is typically malted to prepare micromolecular nutrition for *Saccharomyces cerevisiae* in the later fermentation process. Malting consists of steeping, germination, and kilning stages. Abundant zymogens are activated in the germination, and then catalyze the substance degradation in the malting and the following mashing processes (Mallett, 2014). Conventionally, wheat malt constitutes 5–30% of grains in the high gravity brewing, and no less than 40% for wheat beer.

Unfortunately, Fusarium head blight (FHB) or scab, as a worldwide disease of wheat and barley, is regionally prevalent in China. A range of trichothecenes produced by *Fusariums* were found in wheat and its products, with Deoxynivalenol (DON) or vomitoxin as the most common one (Bianchini et al., 2015; European Food Safety Authority, 2013; Sun & Wu, 2016; Wu, Kuca, Humpf, Klimova, & Cramer, 2017). Noticeably, worldwide surveys of commercial beer have shown DON to be a frequent contaminant (Bertuzzi, Rastelli, Mulazzi, Donadini, & Pietri, 2011; Kostelanska et al., 2009; Varga, Malachova, Schwartz, Krska, & Berthiller, 2013). The high level of DON was detected in wheat beers, a Chinese wheat beer included (Kuzdraliński, Solarska, & Muszyńska, 2013).

It was found that the trichothecene synthase gene (tri5) could be cloned in *F. graminearum*, *F. culmorum*, *F. poae*, *F. sambucinum*, and *F. sporotrichiodes* (Desjardins, 2006). *F. graminearum* species complex is considered to produce DON abundantly and as the most frequently isolated causal agent of FHB in China (Qiu, Sun, Yu, Xu, & Shi, 2016; Zhang et al., 2012). The DON accumulation in the germination of barley was caused by the growth and metabolisms of *Fusariums*, though a portion of DON could be transferred to nontoxic conjugate forms (Habler et al., 2016). However, there is limited information on the development of DON and *Fusariums* during the malting of wheat, except of our updated report on that of

* Corresponding author.
E-mail address: minwei.xu@ndsu.edu (M. Xu).

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hard red spring wheat in America (Jin et al., 2018).

DON contamination occurs severely in the Chinese winter wheat grown in the Yangtze-Huaihe river basin region, which is related to the relatively high rainfall during the period from flowering to harvest. In a survey of 180 wheat samples grown in Jiangsu province of 2010–2012 crop years, DON occurred in 74% of these samples, and the levels ranged from 15 to 41,157 μg/kg with the mean value of 488 μg/kg (Ji, Xu, Liu, Yin, & Shi, 2014). In another survey, DON was detected in 89% of 56 wheat samples harvested from Jiangsu and Anhui provinces in 2010, and the level ranged from 259 to 4975 μg/kg with the mean of 1962 μg/kg (Cui et al., 2013). DON was also found in 95% of the 84 wheat samples from the Yangtze-Huaihe river basin, with the mean value of 3881.2 μg/kg (Selvaraj et al., 2015).

The Chinese government has set the maximum DON limit of 1000 μg/kg in wheat, barley, corn and their flour which are used for human consumption (Chinese Ministry of Health, 2011). Food and Drug Administration (Food and Drug Administration, 2010) in America has set the advisory limit of 1000 μg/kg on processed wheat products, including flour, bran and germ. The European Union has established the maximum permitted level for DON in unprocessed cereals (excluding durum wheat, oats and maize) to 1250 μg/kg, and unprocessed durum wheat and oats to 1750 μg/kg (European Commission, 2006). Brazil legislated it as 1000 μg/kg and 750 μg/kg in whole wheat grain/flour and other wheat derivates, respectively (Silva et al., 2015).

However, it might be hard to adapt these limits in the brewing grains, according to the case that happened in the barley malting, during which *Fusarium* spores in the inner kernel germinated again and produce mycotoxins during the malting under the moderate huminity and temperature conditions (Schwarz, 2017; Wolf-Hall, 2007). DON could increase up to 3-fold following the malting process, around 90% of DON present in the barley malt was transferred to beer by surviving kilning, mashing and fermentation due to its water solubility and thermostability (Dohnal et al., 2010; Schwarz, Casper, & Beattie, 1995). In our newest finding, DON increased by as high as 20-fold in the malting of FHB wheat samples (Jin et al., 2018).

Objective of the study was to evaluate the development of DON and its producing-*Fusariums* in the malting of Chinese winter wheat, which is widely grown in eastern China. The occurrence of DON in wheat in the region is so frequent as mentioned above, and thus, it is hard to have perfectly clean grains without any *Fusarium* infection for beer brewing. In the current study, the wheat samples with DON levels below 1000 μg/kg were selected to malt.

2. Materials and methods

2.1. Samples and quality measurement

Thirteen Chinese winter wheat samples (8 in 2015 crop year, and 5 in 2016) from the Yangtze-Huaihe River basin region were selected to provide a range of DON levels. These samples were provided by an inspection organization in Jiangsu province. The samples were collected after 3 months of harvest, and then frozen at −20 °C before malting.

The quality traits for these wheat samples were tested. Moisture was measured with air-oven method, protein content was measured by Kjeldahl method (N × 6.25), germination rate was determined by placing 100 kernels of wheat on two layers of filter paper with 9.0 cm diameter (Whatman #1) in a petri dish, and adding 4.0 mL of purified water. Germination was control at 20 °C for at most 5 days. Water sensitivity was tested at the same condition but with 8 mL of water added, and defined as the number subtracted from that of 3d germination rate.

2.2. Pilot-scale malting

The conditions for pilot-scale malting was set according to the method for barley (Vegi, Schwarz, & Wolf-Hall, 2011), but some operations were adjusted based one the lab facilities. Each sample (1.20 kg, dry base) was washed by stirring in distilled water for 10 min, and repeated once. All samples were steeped in the uniform container (30 cm × 40 cm × 40 cm) with distilled water at 16 °C. Water was drained out at the intervals of 8 h steeping. Samples were washed once, aerated for 2–3 h in the hollowed-out container, and then re-steeped into clean water for the next steeping stage. After 3 cycles, the steep-out moisture of wheat was between 43 and 45%. Germination was conducted in the basin under the relative huminity of 99% and temperature of 16 °C for 80 h. The green malt was spread on a craft paper, and kilned in the following temperature procedure: 46 °C for 8 h, 57 °C for 6 h, 72 °C for 3 h, and 85 °C for 3 h. Then, rootlets were removed from the kilned malt, and the de-rooted malt was used for DON and *Fusarium* DNA analysis. All the malting was performed in triplicate, and subsamples at steeping out and germination stages were taken in triplicate and freeze-dried before analysis.

2.3. DON analyses

DON was analyzed by the Chinese national standard method of HPLC coupled with clean-up immunoaffinity column (GB 5009.111–2016), with the modification of DON extract procedure. Samples were ground using a coffee grinder to fine powder. Each ground sample (25 g) was extracted with 100 mL of acetonitrile/water solvent (84/16, v/v) by shaking on a laboratory shaker at 150 r/min for 1 h. After filtering through Whatman glass fiber filter paper (Whatman PLC, Maidstone, UK) of 70 mm diameter, the filtrate was collected. Extract (5 mL) was added to an immunoaffinity column (VICAM DONtest WB, MO, America) according to the instruction. The eluate was collected and evaporated to dryness at 55 °C for around 60 min. The residue was re-dissolved into 0.5 mL of mobile phase of HPLC, and filtered through a 0.45 μm filter paper (EMD Millipore, Billerica, MA, USA).

The HPLC equipment (Agilent 1100) with a UV detector was employed in the study. Separation was performed with a ODS column (5 μm, 250 × 4.6 mm, Agilent, CA, USA), at the temperature of 20 °C. The mobile phase was a mixture of water/methanol/acetonitrile (90/6/4, v/v/v), with a flow rate of 1.0 mL/min. The injection volume was 20 μL. DON was detected under the wavelength of 220 nm. The limit of detection (LOD) and quantification (LOQ) for DON were 50 μg/kg and 100 μg/kg, respectively.

2.4. Real-time quantitative PCR

A portion (50 mg) of each fine ground sample was weighted out for DNA extraction. Wheat genomic DNA was extracted by a GenElute Plant Genomic DNA miniprep kit (Sigma-Aldrich, Milwaukee, WI, USA) with protocol as described. An CFX Connect ™ Real-time Detection System (Bio-Rad, Hercules, CA, USA) was used to run real-time quantitative PCR (qPCR). The *Fusarium* DNA of trichothecene producing species was measured by amplifying a fragment (178 bp) on the tri5 gene, and the qPCR procedure was carried out according to the description by Brunner et al. (2009). For quantification, genomic *F. granimearum* DNA (50 ng, 5 ng, 500 pg, 50 pg and 5 pg) was used to derive a calibration curve.